—Review—

The nuclear phase of human oocytes during ICSI and nuclear transfer procedures

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Abstract: Human oocytes have the aggregated chromosome phase (AC phase) during the first and second meiosis. This needs to be better understood, as the timing of ICSI significantly influences ART outcomes. In fact, performing ICSI after the completion of MII spindle formation is known to improve successful fertilization and embryo development. This human AC phase should also be taken into consideration in the application of nuclear transfer/mitochondrial replacement for patients suffering from severe mitochondrial diseases, to prevent the transmission of these diseases to their offspring, with the aim of limiting the risk of mitochondrial carryover. The possible risks and benefits of AC transfer and other procedures for mitochondrial replacement are reviewed and discussed in this paper.

Key words: ICSI, Aggregated chromosome, Nuclear transfer, Mitochondrial replacement

Introduction

Animal studies have contributed greatly to the evolution of assisted reproductive technology in humans, however, human oocytes have unique characteristics. For instance, human oocytes frequently exhibit some dysmorphic features such as a tubular type of smooth endoplasmic reticulum cluster [1], refractile bodies/lipofuscin bodies [2], and centrally located cytoplasmic granularity [3], which have not been observed in other species apart from the chimpanzee [4]. Furthermore, in human germinal vesicle (GV) stage oocytes, following the completion of nucleolar breakdown, chromosomes are assembled in a single aggregation that heralds the start of nuclear membrane breakdown. In contrast, nucleolar and nuclear membranes in mouse GV stage oocytes, start to break down almost simultaneously [5].

In this article, the nuclear phases during meiosis in human oocytes, which must be better understood to improve ART outcomes, are reviewed and discussed. In addition, some critical points, which should be addressed when nuclear transfer technology is applied to humans, are also reviewed and discussed.

The Aggregated Chromosome Phase during Meiosis and the Optimal Timing of ICSI in Human Oocytes

A higher rate of chromosomal abnormality is observed in human oocytes as compared to other animal oocytes, and the cause of this phenomenon is not yet clearly understood. We previously reported that human oocytes have an aggregated chromosome phase (AC phase) during the first and second meiosis, and this nuclear phase has not been observed in other species [5]. In this AC phase, chromosomes are aggregated before the formation of spindle microtubules (Fig. 1). This is consistent with the finding that a spindle completely disappears for approximately 40–60 min during the transition from metaphase I to metaphase II, when oocytes are observed by a polscope [6]. Yu *et al.*, investigated the optimal timing of ICSI in relation to oocyte maturation and reported that oocytes with a normal spindle assembly, in the 2–5 h after the first polar body extrusion, give rise to good quality embryos at a significantly higher rate [7]. Kilani *et al.*, reported that the average MII spindle retardance observed by polscope peaked at 39–40.5 h after hCG administration [8]. The fertilization rate of oocytes within 1–1.5 h after the first PB extrusion was reported to be low [9, 10] due to the fact that all the oocytes within 1 h after PB extrusion were at telophase I [10]. The results of these studies suggest that the timing of ICSI significantly influences the outcome, and that performing ICSI

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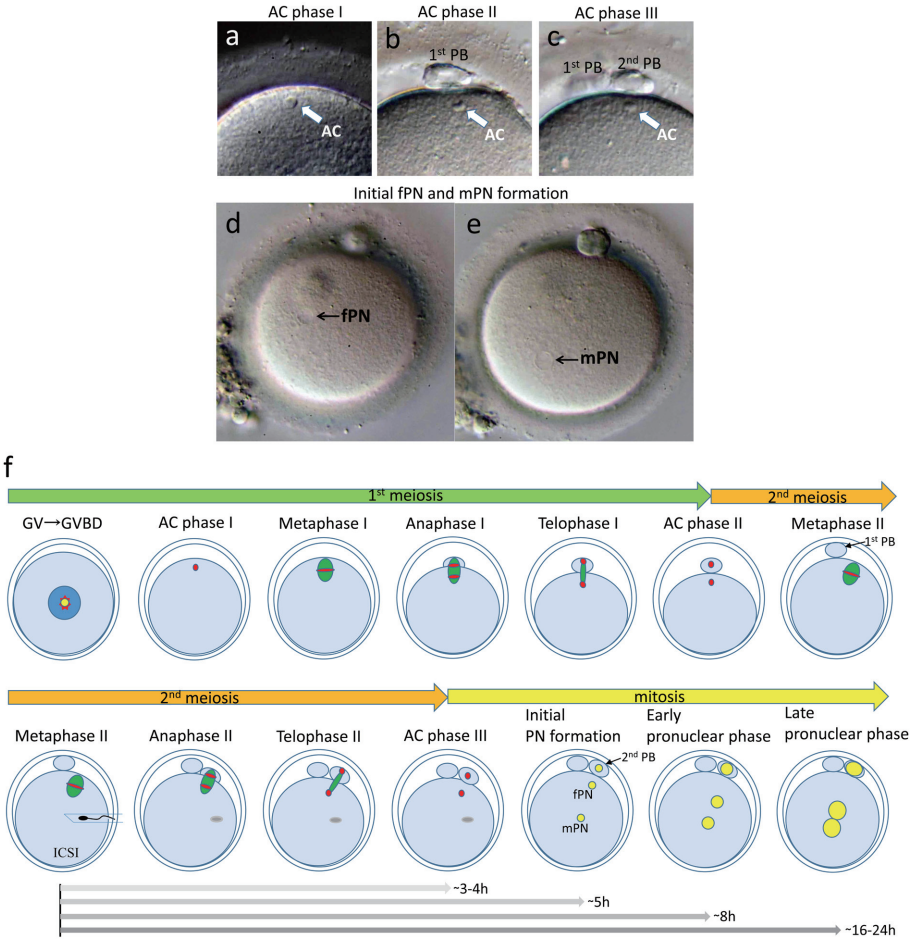

Fig. 1. Aggregated chromosome phase (AC phase) during the first and second meiosis in human oocytes. (a) Aggregated chromosome phase I (AC phase I) during which chromosomes assemble in a single aggregation following germinal vesicle breakdown (GVBD). (b) Aggregated chromosome phase II (AC phase II), between telophase I and metaphase II. (c) Aggregated chromosome phase III (AC phase III) between telophase II and initial pronuclear formation. (d) The beginning of female pronuclear formation, which occurs shortly after AC phase III. (e) The beginning of male pronuclear formation in the same zygote, photographed at the same time as Fig 1-d. (f) Progression from GVBD to completion of 2PN formation, showing the approximate length of time from ICSI to AC phase III, from ICSI to initial PN formation, from ICSI to early pronuclear phase and from ICSI to late pronuclear phase, consecutively.

when the MII spindle is already formed can result in successful fertilization and embryo development. Although polarized microscopy is useful for confirming the presence and position of the MII spindle, the chromosomal status during a transitional period such as the AC phase cannot be identified using polarized microscopy. Therefore, when aggregated chromosomes are detected using differential interference contrast (DIC) microscopy, the oocyte culture should be extended until a metaphase II spindle is formed.

Considering the "Aggregated Chromosome Phase" in the Application of Nuclear Transfer Technology to Humans

Nuclear transfer/mitochondrial replacement in humans has been approved in the UK for patients suffering from severe mitochondrial diseases, to prevent the transmission of these diseases to offspring [11]. It has been considered that 1 in 10,000 people have clinically manifest mtDNA diseases [12] and 1 in 200 harbor pathogenic mtDNA mutations [13].

Table 1. Advantage and disadvantage of nuclear transfer in each procedure

	Cytochalasine B/ DMAP* for DNA extraction	Electrofusion/ sendai virus for cell fusion	Stage of door oocytes	Time lapse observation	Mitochondria carryover
Germinal vesicle transfer (GVT)	Required	Required	GV donor oocytes	Not required	GVT, PNT>MSCT, PBT>>ACT,fPNT
MII spindle chromosome complex transfer (MSCT)	Required	Required	MII donor oocytes	Not required	
Pronuclear transfer (PNT)	Required	Required	MII donor oocytes	Not required/ Useful	
PB transfer (PBT)	Not required	Required	MII donor oocytes	Not required	
Aggregated chromosome transfer after 1st PB extrusion (ACT1)	Not required/Low dose	Not required	GV/MI donor oocytes	Required	
Aggregated chromosome transfer after 2nd PB extrusion (ACT2)	Not required/Low dose	Not required	MII donor oocytes	Required	
Female pronuclear transfer in early PN stage (fPNT)	Not required/Low dose	Not required	MII donor oocytes	Required	

*DMAP: N-(4-Pyridyl) dimethylamine

As mitochondria play an important role in the energy generation of cells and cellular metabolic functions, dysfunction in mitochondria and mtDNA mutation can cause damage to the brain cells, the heart, liver, muscles, kidneys and the respiratory and endocrine systems, depending upon which cells are affected [14–17]. These dysfunctions and mutations are often life-threatening. Possible preventative solutions include adoption, oocyte/embryo donation, preimplantation genetic diagnosis (PGD) and nuclear transfer. However, most patients are eager to pass their own DNA on to their offspring, thus, the latter two options may currently be possible solutions for these patients. PGD involves the selection of an embryo below the threshold of mitochondrial mutation, which is considered to be 18% [18]. Therefore PGD, finding embryos with no mtDNA mutation, is not always possible. In addition, PGD is not suitable for patients with a homoplasmic mutation. Nuclear transfer would therefore be the only therapeutic option for these patients.

Germinal vesicle transfer (GVT) [19], MII spindle-chromosome complex transfer (MST) [20, 21], pronuclear transfer (PNT) [22, 23], polar body transfer (PBT) [24] and aggregated chromosome transfer (ACT) [25] have been reported as procedures for nuclear transfer. Regarding ACT, it can also be performed after the oocytes have been fertilized, as aggregated chromosomes (AC) can also be observed after the second polar body extrusion [5, 25]. The AC phase lasts for only 0.5–3 h, during which a small female pronucleus (fPN) is formed and the size of the fPN gradually increases until the pronuclear membrane breaks down [26]. As the fPN is localized near the second polar body in this early phase, it is easy to distinguish from a male PN. The AC can be extracted with only a minimal amount of ooplasm, which is a much smaller amount of ooplasm than in GVT, PNT, MST and PBT. The transfer of AC could allay the concern about heteroplasmy as it limits the carryover of pathological mitochondria. As the AC in human oocytes can be clearly seen using DIC microscopy and the aggregate can be removed and injected using an ICSI injection pipette [25], damage to the ooplasm is minimal, as in ICSI. Furthermore, it is possible to avoid the use of cytochalasin B when AC are removed from both recipient and donor oocytes. AC transfer is a less harmful procedure and does not require electrical fusion or cell fusion using Sendai virus [25]. One disadvantage of ACT and fPNT is that the durations of the AC and small fPN phase are short (0.5–3 h) and must be observed using a time-lapse system (Table 1). The AC transfer after the first polar body extrusion (ACT II) and the second polar body extrusion (ACT III), and fPN transfer (fPNT) are described in Fig. 2

Reported Birth after NT/MR in Humans

Zhang J *et al.* reported on a pregnancy derived from human pronclear transfer performed in 2003 for a 30 year-old patient who had two consecutive failures due to all of her embryos being arrested at the two-cell stage [27]. For her third IVF cycle, PNT was performed and five viable reconstructed embryos were transferred to the pa-

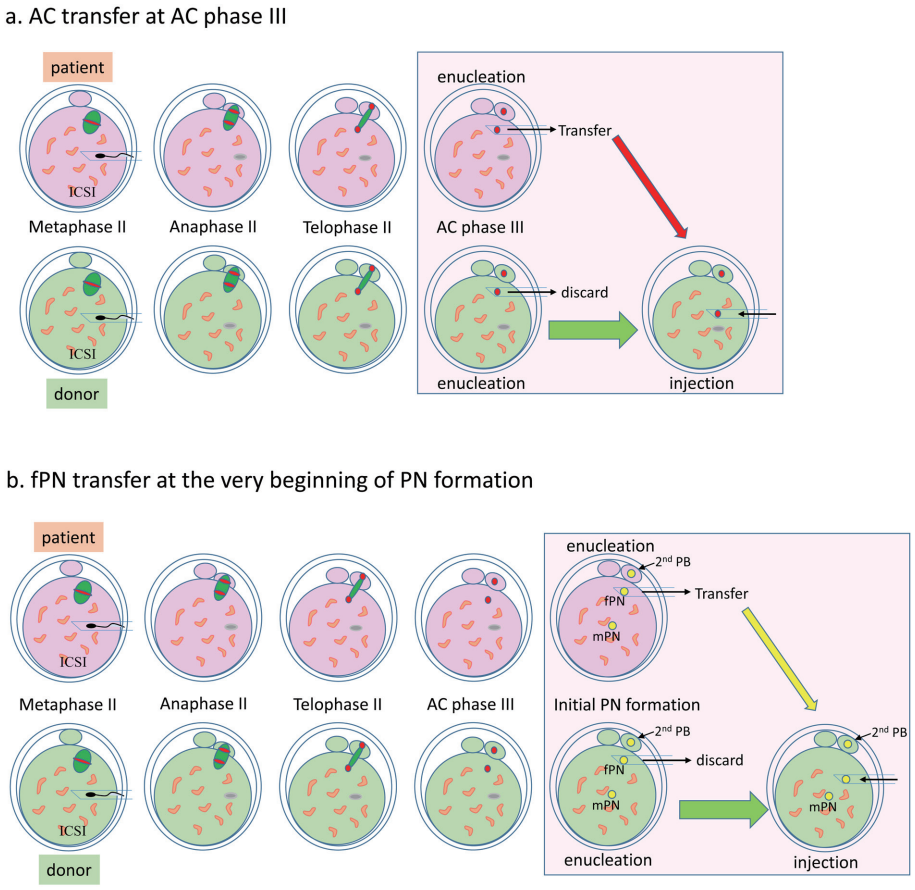

Fig. 2. Nuclear transfer / mitochondria transfer at AC phase III and the very beginning of fPN formation. (a) Schematic diagram of aggregated chromosome transfer during AC phase III, depicting a proposed new chromosome replacement procedure, from oocytes containing abnormal mitochondria into oocytes of donors containing normal mitochondria. (b) Schematic diagram of fPN transfer at the very beginning of PN formation, depicting another proposed new procedure.

tient's uterus, which resulted in a triplet pregnancy. Fetal reduction from a triplet to twin pregnancy was performed. At 24 weeks, the second fetus was delivered as a result of a premature membrane rupture, and did not survive. The third fetus was delivered after intrauterine fetal demise at 29 weeks due to cord prolapse. They reported that all of the delivered babies had normal karyotypes and that the fetal mitochondrial DNA profiles were identical to those of the donor cytoplasm. The patient's mitochondrial DNA was not detected.

Risks of NT/MR

One of the major concerns regarding NT/MR is mitochondrial carryover. It has been reported that no recipient mitochondria were detected in embryonic stem cells derived from spindle–chromosome complex-transferred oocytes [28]. However, Yamada *et al.* reported that even though low levels of heteroplasmy introduced into human oocytes by mitochondrial carryover during nuclear transfer often vanish, they can sometimes result in mtDNA genotypic drift and reversion to the original genotype [29]. Furthermore, embryos produced by MT/MR will acquire mtDNA from the female donor, resulting in genetic material from three different individuals. These novel combinations of genetic material may not be fully compatible with one another [30]. It should also be noted that there may be unknown risks of NT/MR, as mitochondria-nuclear interactions are not yet entirely clear. Epigenetic changes with NT/MR are a further concern and have also yet to be studied.

Future Directions and Conclusion

In this article, some of the specific features of human oocytes were reviewed and human-specific procedures for NT/MR were introduced. Experimental results suggest that ACT II, III and fPNT could be used as procedures to reduce the risk of heteroplasmy and the possible negative effects of the use of chemicals and artificial cell fusion. Although the birth of some babies with PNT has been reported, no risk analysis has yet been carried out in the UK, the US, or China. Risk analysis is urgently required before nuclear transfer is clinically performed.

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